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Anne Holleran
AU: 1642
Tel: 308-8892
RM: 8e03

mailbox: 8e12

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# Effects of Tumor Necrosis Factor-α on the In Vitro Maturation of Tumor-Reactive Effector T Cells

*Keishi Tanigawa, †Ronald A. Craig, †Lloyd M. Stoolman, and *Alfred E. Chang

*Division of Surgical Oncology and †Department of Pathology, University of Michigan, Ann Arbor, Michigan, U.S.A.

**Summary:** Culture methods that enhance the anti-tumor reactivity of primed T cells would be important in adoptive immunotherapy of cancer. Using several different syngeneic murine tumor models, the authors evaluated the effects of tumor necrosis factor-α (TNF-α) exposure on tumor-draining lymph node (TDLN) cells during in vitro activation. Mice were inoculated with weakly immunogenic (i.e., MCA 205, MCA 207 sarcoma) or the poorly immunogenic (i.e., D5 melanoma) tumor cells, and TDLN cells were harvested 9 or 10 days later for activation by an anti-CD3/interleukin-2 culture procedure. Human recombinant TNF-α (25 ng/mL) added during the activation culture resulted in a two-fold increase in interferon-γ release (type 1 response) and a significant reduction of interleukin-10 (type 2 response) after tumor antigen stimulation. In an adoptive transfer model, TNF-α–cultured TDLN cells mediated significantly greater regression of established tumor than did TDLN cells cultured in the absence of TNF-α in five of five experiments. Neutralization of interleukin-10 monoclonal antibody further augmented the therapeutic efficacy of TNF-α–cultured TDLN cells. These studies document the ability of TNF-α to selectively promote a type 1 over a type 2 response in a bulk population of tumor-primed T cells during in vitro activation. **Key Words:** Adoptive immunotherapy—Cytokines—Vaccines—Neoplasms.

Several studies have documented the efficacy of adoptively transferred immune cells in mediating the regression of established metastases in animal and clinical studies (1–5). One approach we have focused on is the retrieval of tumor-reactive T cells from either tumor-draining lymph nodes (TDLN) or vaccine-primed lymph nodes (6–10). In murine models, we have reported that $CD8^+$ TDLN cells secondarily activated with anti-CD3 monoclonal antibody (mAb) (anti-CD3) and interleukin (IL)-2 mediate immunologically specific tumor regression. Generating more effective TDLN cells on a per-cell basis would have potential clinical usefulness.

We previously reported that type 1 and type 2 cytokine profiles are released by T cells in response to tumor antigen and predict the therapeutic ability of T cells to mediate tumor regression (11). In our murine studies, $CD8^+$ T cells with type 1 cytokine release profiles to tumor antigen were responsible in mediating tumor regression. In contrast, $CD8^+$ cells that secreted IL-10, a type 2 cytokine, were associated with suppression of anti-tumor reactivity. Therefore, methods at enhancing type 1–expressing $CD8^+$ T cells would be clinically relevant.

Tumor necrosis factor-α (TNF-α) is a multifunctional cytokine produced mainly by activated macrophages, T cells, mast cells, and some endothelial cells. A wide range of biologic effects are elicited by TNF-α, including hemorrhagic necrosis of transplanted tumors; growth proliferation of normal cells; cytotoxicity; and inflammatory, immunoregulatory, and antiviral responses (12–15). In addition, TNF-α has immunomodulating effects that enhance host immune responses to tumor (16–19). Interleukin-4 also regulates a wide range of biologic ac-

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Address correspondence and reprint requests to Dr. Alfred E. Chang, 3302 Cancer Center, 1500 E. Medical Center Drive, Ann Arbor, MI 48109, U.S.A.

tivities, including the activation, growth, and differentiation of all hematopoietic lineages (20). One of the critical effects that IL-4 has been reported to mediate is the stimulation of type 2 responses of T cells, which results in the secretion of a panoply of cytokines, including IL-4, IL-5, IL-10, and IL-13 (20–23). One mechanism by which poorly immunogenic tumors fail to elicit an immune response is postulated to be the induction of a type 2 host response (24).

In this study, we examined the divergent effects that TNF-α and IL-4 have on the maturation of TDLN cells during in vivo activation. The effect these cytokines have on the functional properties of pre-effector T cells may play a role in the complex balance of factors that occur during the in vivo response of the host to progressive tumors. Furthermore, our findings indicate that culture conditions can be defined, in the absence of tumor antigen, that can upregulate the maturation of tumor-reactive T cells.

## MATERIALS AND METHODS

### Mice

Female C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and maintained in a specific pathogen-free environment. They were used after 8 weeks of age. Recognized principles of laboratory animal care were followed, and all animal protocols were approved by the University of Michigan Laboratory of Animal Medicine.

### Tumors

The B16-BL6 melanoma is a spontaneously occurring tumor that has been studied extensively (25). A subclone of the B16-BL6 tumor, termed D5, has been characterized previously by our laboratory (26). Tumor cells were maintained by serial in vitro passage in complete medium (CM), which consisted of RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 0.01 μmol/L nonessential amino acids, 1 μmol/L sodium pyruvate, 2 μmol/L fresh L-glutamine, 100 mg/mL streptomycin, 100 IU/mL penicillin, 50 mg/mL gentamicin, 0.5 mg/mL fungizone (all reagents from GIBCO, Grand Island, NY), and $10^{-5}$ mol/L 2-mercaptoethanol. For intravenous and subcutaneous tumor inoculation, adherent tumor cells were removed from the culture flasks using trypsin and washed in CM. Tumor cells were then washed twice in Hank's balanced salt solution for administration into mice or resuspended in CM for in vitro assays.

MCA 205 and MCA 207 are syngeneic fibrosarcoma cell lines from C57BL/6 mice induced by 3-methylcholanthrene and previously characterized as immunologically distinct (27). They were provided by Dr. James C. Yang (National Cancer Institute, National Institutes of Health, Bethesda, MD). The sarcoma tumors were maintained in vivo by serial subcutaneous transplantation in syngeneic mice and were used before the seventh passage. Tumor cell suspensions were prepared from solid tumors by enzymatic digestion in 40 mL Hank's balanced salt solution containing 40 mg/mL collagenase, 4 mg/mL deoxyribonuclease I, and 100 units/mL hyaluronidase (Sigma Chemical, St. Louis, MO) for 3 hours at room temperature, as previously described (9). Tumor cells were washed three times in Hank's balanced salt solution for administration into mice or resuspended in CM for in vitro assays.

### Tumor-Draining Lymph Nodes

A quantity of $10^6$ tumor cells suspended in 0.05 mL Hank's balanced salt solution was inoculated subcutaneously into the lower flanks of syngeneic B6 mice. Nine or 10 days after tumor inoculation, TDLN cells were harvested under sterile conditions. Lymph nodes were dissociated into single-cell suspension by teasing apart the TDLN cells using 20-gauge needles and then pressing the tissue fragments with the blunt end of a 10-mL plastic syringe. Connective tissue was removed using nylon mesh, and the lymphocytes were resuspended in CM for in vitro activation.

### In Vitro Activation

Tumor-draining lymph node cells were activated for 2 days in anti-CD3 mAb (anti-CD3) immobilized in 24-well culture plates at $4 \times 10^6$ cells/well. Anti-CD3 is a hamster mAb directed against the CD3Ω chain of the murine T-cell antigen receptor CD3 complex. The hybridoma (2C11) was provided by Dr. Jeffrey Bluestone (University of Chicago, Chicago, Illinois). After anti-CD3 activation, the cells were harvested from the culture plates and resuspended at $2 \times 10^5$ cells/mL in CM containing 60 IU/mL IL-2 ($18 \times 10^6$ IU/mg protein). Human recombinant IL-2 was provided by Chiron Therapeutics (Emeryville, CA). In this study, international units (IU) are expressed in all of the experiments.

### Recombinant Human Tumor Necrosis Factor-α and Murine IL-4

Human recombinant TNF-α was provided by Dr. Douglas Fraker (University of Pennsylvania, Philadel-

phia, PA) at a specific activity of $8.2 \times 10^6$ U/mg protein. Sterile, lyophilized TNF-α (0.79 mg/vial) was first reconstituted with 1 mL sterile water before further dilution in culture medium and stored in a −20°C freezer until use. In some experiments, murine IL-4 was also used in the same manner. Recombinant murine IL-4 was obtained from Becton Dickinson (San Diego, CA).

### Adoptive Immunotherapy

B6 mice were inoculated intravenously with $3 \times 10^5$ tumor cells to establish pulmonary metastases. Three days after tumor inoculation, mice were injected intravenously with activated TDLN cells. Approximately 2 to 3 weeks after tumor inoculation, mice were killed so pulmonary metastatic nodules could be counted. The MCA 205 and MCA 207 lung nodules appear as discrete white nodules on the black surface of lungs insufflated with a 15% solution of black India ink and fixed by Fekete solution (28). In contrast, D5 lung metastases appear as black nodules on lungs insufflated with Fekete solution. Metastatic foci too numerous to count were assigned a value of >250. The significance in the mean number of pulmonary metastases between experimental groups was determined using the nonparametric Wilcoxon rank-sum test. Two-sided probability values $\leq 0.05$ were considered significant. Each group consisted of at least five mice, and no animal was excluded from the statistical evaluation.

### Immunofluorescence and Flow Cytometric Analysis

After cell-surface staining, FITC conjugated anti-mouse interferon-γ (IFN-γ) and phycoerythrin (PE)-conjugated anti-mouse IL-10 (both from PharMingen, San Diego, CA) were used to detect intracytoplasmic cytokine (29). Briefly, TDLN cells were activated in vitro for 2 days with anti-CD3 mAb and then for 3 days in human IL-2. Further activation was achieved and intracellular transport was blocked by incubation of the cells for 6 hours with 50 ng/mL phorbol myristate acetate, 1 μg/mL ionomycin, and 2 μmol/L monensin. The cells were stained with rat anti-mouse CD8a-CyChrome (PharMingen)and then fixed and held overnight at 4°C in 4% paraformaldehyde solution. Cells were permeabilized by washing twice in 0.1% saponin. Either anti–mIFN-γ fluorescein isothiocyanate or anti–mIL-10-PE was added to the permeabilized cells. An isotype-matched control (rat IgG2a-CyChrome) was used to assess nonspecific staining by the CD8 mAb. Nonspecific staining for the cytokines was determined by preincubation of the cells with a 50-fold excess of unlabeled cytokine mAb 30 minutes before the addition of labeled mAb. Analysis was conducted using a Becton Dickinson FACScan and Winlist Listmode program (Verity Software, Topsham, MA). Fluorescence profiles were generated by analyzing 10,000 cells and displayed as logarithmically increasing fluorescence intensity versus cell numbers.

### Measurement of In Vitro Cytokine Release

After in vitro activation, TDLN cells were restimulated with irradiated autologous tumor cells in CM or with immobilized anti-CD3. Tumor stimulator cells (MCA 205 or MCA 207) were irradiated to 7,000 cGy by a $^{137}Cs$ source before use. In lieu of D5 as stimulator cells, anti-CD3 stimulation was used to detect cytokine. One million TDLN cells and the same number of irradiated tumor cells were cocultured in 2 mL CM with 60 IU rhIL-2 in 24-well tissue culture plates. After 48 hours, culture supernatants were collected for cytokine measurement and measured in duplicate wells using commercially available enzyme-linked immunosorbent assay kits. If detectable, background cytokine values produced by tumor cells alone or lymphocytes alone were subtracted from the mixed lymphocyte tumor cell cultures.

### In Vivo Neutralization of Murine IL-10

Inhibition of IL-10 activity in vivo was performed by the intravenous administration of murine IL-10–neutralizing mAb. Anti–IL-10 mAb was made from hybridoma JESS-2A5.1.1 obtained from the American Type Culture Collection (Manassas, VA). Hybridoma cells were cultured in CM and injected intraperitoneally ($5 \times 10^7$ cells) into cyclophosphamide-pretreated DBA/2 mice after 500 cGy whole-body irradiation. Approximately 8 days later, ascites were harvested and centrifuged to remove remaining cells. The efficacy of the neutralizing activity was measured by enzyme-linked immunosorbent assay, in which a 1:1000 ascites dilution of fluid could neutralize at least 20 ng/mL IL-10. In the adoptive transfer model, 0.2 mL ascites was administered into the caudal vein daily for 4 days after cell transfer. Control groups were given 0.25 mg rat immunoglobulin (RIg) intravenously at the same dose and intervals as the IL-10–neutralizing mAb.

## RESULTS

### Effects of Tumor Necrosis Factor-α or IL-4 During the Activation of D5 Tumor-Draining Lymph Node Cells

We examined the immune function of D5 TDLN cells exposed to TNF-α or IL-4 during in vitro activation.

Tumor necrosis factor-α (25 ng/mL) or IL-4 (50 μ/mL) were added to anti-CD3 activation cultures and during the expansion period in IL-2. The TDLN cells were subsequently harvested and washed for in vitro cytokine release assays. Activated D5 TDLN cells were secondarily stimulated with anti-CD3 and the supernatants harvested 24 hours later for cytokine measurement. As illustrated in Figure 1, TDLN cells exposed to TNF-α during activation secreted more IFN-γ and less IL-10 than control TDLN cells. In contrast, TDLN cells cultured with IL-4 resulted in decreased release of IFN-γ and increased release of IL-10.

Intracytoplasmic staining of D5 TDLN cells after exposure to TNF-α or IL-4 during in vitro activation was assessed with anti–IFN-γ and anti–IL-10 mAbs. As shown in Figure 2, intracytoplasmic IFN-γ staining of D5 TDLN cells was enhanced after exposure to TNF-α. In contrast, these levels of intracytoplasmic IFN-γ were suppressed in D5 TDLN cells after exposure to IL-4. Intracytoplasmic IL-10 was unchanged by TNF-α exposure but was increased four times compared with standard culture conditions with exposure to IL-4. A ratio of intracytoplasmic IFN-γ to IL-10 using the areas under each histogram for positively stained cells was calculated. Using this method, the ratios of IFN-γ to IL-10 for standard, TNF-α–cultured, and IL-4–cultured D5 TDLN cells were 4, 6.8, and 1, respectively.

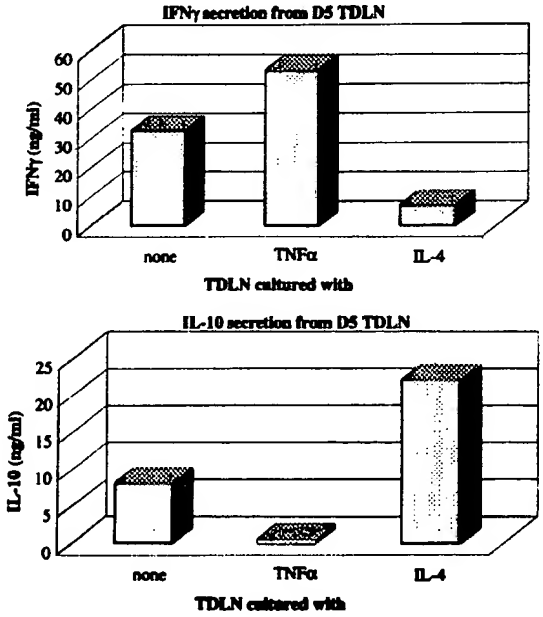


FIG. 1. Interferon-γ and IL-10 release of D5 TDLN cells in response to anti-CD3 stimulation. Before assessment of cytokine release, TDLN cells were cultured by the anti-CD3 and IL-2 method in the presence of TNF-α, IL-4, or no added cytokine (none).

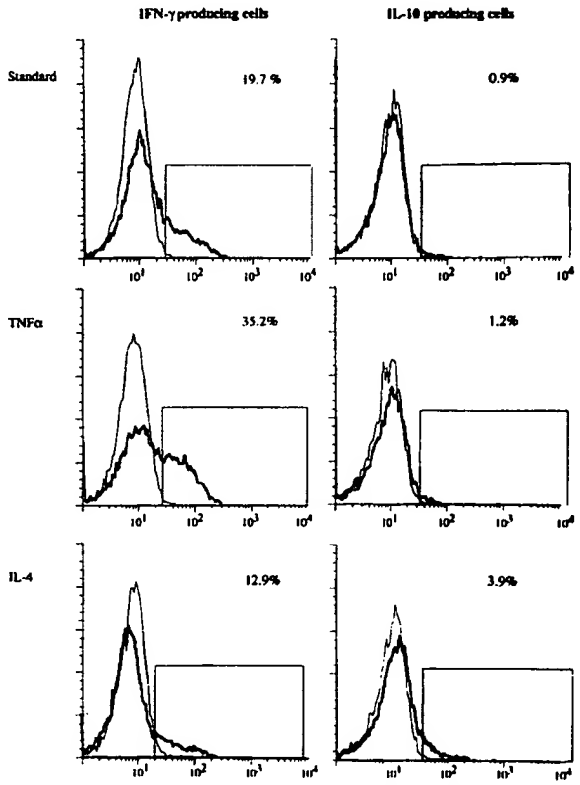


FIG. 2. Intracytoplasmic IFN-γ and IL-10 staining of D5 TDLN cells after anti-CD3 and IL-2 activation in the presence of TNF-α, IL-4, or no added cytokine (standard).

We also examined the in vivo anti-tumor reactivity of the D5 TDLN cells in adoptive immunotherapy experiments. The TDLN cells cultured in TNF-α or IL-4 were adoptively transferred to mice with 3-day-old pulmonary metastases. As summarized in Table 1, $4 \times 10^7$ TNF-α–cultured TDLN cells mediated significant reduction of pulmonary metastases compared with control TDLN cells in two of two experiments. Control TDLN cells failed to mediate tumor regression, which we have observed in previous studies, and underscored the poorly immunogenic characteristics of D5 tumor cells (8,26). As expected, exposure of TDLN cells to IL-4 did not yield therapeutic effector T cells (see Experiment 1 in Table 1).

## Effects of Tumor Necrosis Factor-α on the Activation of MCA 207 Tumor-Draining Lymph Node Cells

To confirm the augmentation of TNF-α on TDLN cell activation, we used a tumor with different immunologic characteristics, the MCA 207 sarcoma. Unlike D5 mela-

**TABLE 1.** *In vivo efficacy of D5 TDLN stimulated with IL-4 or TNFα*

| Adoptive Immunotherapy* | | | |
|---|---|---|---|
| TDLN cells cultured with† | No. Cells | Mean No. Lung Mets (SEM) Exp 1 | Exp 2 |
| A. — | None | >250 | 247 (3) |
| B. Standard | $4 \times 10^7$ | >250 | 196 (30) |
| C. Standard | $1 \times 10^7$ | >250 | 229 (14) |
| D. TNFα | $4 \times 10^7$ | 91 (11)‡ | 76 (26)‡ |
| E. TNFα | $1 \times 10^7$ | 195 (25) | 201 (26) |
| F. IL-4 | $4 \times 10^7$ | 234 (12) | N.D. |
| G. IL-4 | $1 \times 10^7$ | >250 | N.D. |

TDLN, tumor-draining lymph nodes; TNF, tumor necrosis factor; SEM, standard error of the mean; N.D., not done.

* Mice were inoculated with $3 \times 10^5$ tumor cells intravenously. Three days later, some groups of mice received effector cells at the indicated dose. All groups of mice received IL-2 60,000 IU intraperitoneally daily commencing after cell transfer for eight doses. Lungs were harvested on day 14.

† Nine days after D5 tumor subcutaneous inoculation, TDLN were harvested for *in vitro* activation by the anti-CD3/IL-2 method with or without additional TNFα (25 ng/mL) or IL-4 (50 U/mL). Group A represents groups of mice that received no TDLN cells.

‡ $p < 0.0001$ compared to A and B.

noma, which does not express major histocompatibility complex class (MHC) I or II molecules (30), the MCA 207 expresses MHC class I and was previously characterized as "weakly" immunogenic (31). As previously reported, MCA 207 TDLN cells activated by the anti–CD3 and IL-2 method generates immune effector T cells that can mediate tumor regression in adoptive immunotherapy experiments (32). In the current studies, MCA 207 TDLN cells were activated in the presence of TNF-α (25 ng/mL) and subsequently evaluated for cytokine release in response to irradiated MCA 207 tumor cells. As illustrated in Figure 3, TNF-α–cultured TDLN cells resulted in enhanced IFN-γ and reduced IL-10 release in response to tumor stimulation.

Next we examined the efficacy of TNF-α–cultured MCA 207 TDLN cells in the adoptive immunotherapy of 3-day-old pulmonary metastases. As summarized in Table 2, TNF-α–cultured TDLN cells mediated significantly greater tumor regression than did control TDLN cells in two of two experiments.

## Effect of IL-10 Neutralization on the Anti-Tumor Reactivity of Tumor Necrosis Factor-α–Cultured Tumor-Draining Lymph Node Cells

From the studies reviewed here, we have shown that TNF-α can upregulate type 1 (i.e., IFN-γ) and downregulate type 2 (i.e., IL-10) responses of activated TDLN cells. To further evaluate the role of type 2 reactivity of

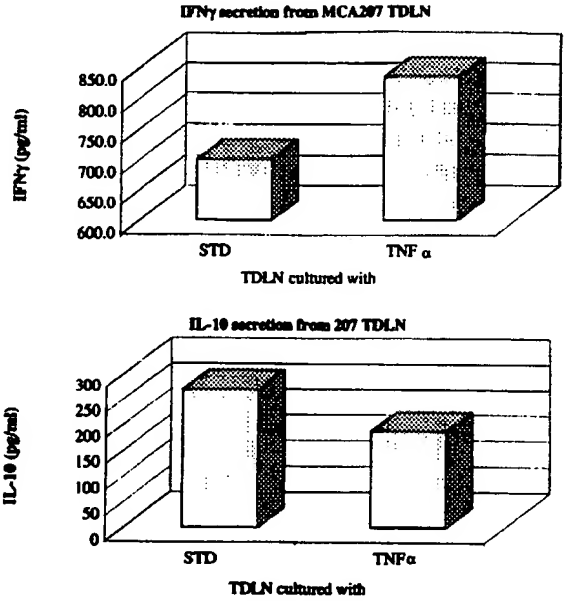


**FIG. 3.** Interferon-γ and IL-10 release of MCA 207 TDLN cells in response to irradiated tumor cells. Before assessment of cytokine release, TDLN cells were activated by the anti-CD3 and IL-2 method in the presence of TNF-α or in its absence (STD).

TDLN cells, we inhibited the biological effects of IL-10 by administering neutralizing mAb in an adoptive immunotherapy experiment. Using the MCA 205 sarcoma, another weakly immunogenic tumor that is immunologically distinct from MCA 207, we activated TDLN cells with or without TNF-α. In a cytokine-release assay, TNF-α–cultured MCA 205 TDLN cells released more

**TABLE 2.** *In vivo efficacy of MCA207 TDLN stimulated with TNFα*

| Adoptive Immunotherapy* | | | |
|---|---|---|---|
| TDLN cells cultured with† | No. cells | Mean No. Lung Mets (SEM) Exp 1 | Exp 2 |
| A. — | None | 134 (21) | 202 (16) |
| B. Standard | $2 \times 10^6$ | 91 (15) | 134 (29) |
| C. TNFα | $2 \times 10^6$ | 41 (10)‡ | 76 (23)§ |

TDLN, tumor-draining lymph nodes; TNF, tumor necrosis factor; SEM, standard error of the mean.

* Mice were inoculated with $3 \times 10^5$ tumor cells intravenously. Three days later, some groups of mice received effector cells. All groups of mice received IL-2 60,000 IU intraperitoneally daily commencing after cell transfer for eight doses. Lungs were harvested on day 18 or 21.

† Nine days after MCA 207 tumor subcutaneous inoculation, TDLN were harvested for *in vitro* culture by the anti-CD3/IL-2 method with or without additional TNFα (25 ng/mL).

‡ $p < 0.05$ compared to A and B.

§ $p < 0.05$ compared to A and B.

IFN-γ than did control TDLN cells in response to MCA 205 tumor cells but still elaborated IL-10, as shown in our previous experiments (data not shown). The activated cells were transferred into mice with 3-day-old pulmonary metastases. Some groups of animals received anti–IL-10 mAb, whereas others received control rat immunoglobulin. As summarized in Table 3, anti–IL-10 mAb administration also had no effect on the reduction of pulmonary metastases. As shown previously, TNF-α–cultured TDLN cells mediated significantly greater anti-tumor effects than TDLN cells cultured under standard conditions. The administration of anti–IL-10 mAb significantly enhanced the anti-tumor reactivity of either standard or TNF-α–cultured TDLN cells. With the neutralization of IL-10, the TNF-α–cultured TDLN cells were significantly more effective in mediating tumor regression than were standard TDLN cells. This suggested that the augmented type 1 response induced by TNF-α is associated with the in vivo therapeutic efficacy of adoptively transferred cells.

## DISCUSSION

The adoptive transfer of appropriately sensitized T cells has been reported to mediate the regression of advanced tumor burdens in animal models (3,6). Initial clinical studies have shown significant tumor responses in a subgroup of patients with metastatic disease (10,33). An important focus of our laboratory efforts has been to devise methods to increase our ability to generate tumor-reactive effector T cells from lymphoid sources of patients to increase the effectiveness of such therapy. Based on preclinical murine studies, we found that lymph nodes draining inocula of tumor cells or vaccine agents serve as a reliable source of "pre-effector T cells" (7,34). In the freshly harvested state, these pre-effector cells are not functionally able to mediate anti-tumor responses in adoptive immunotherapy experiments. However, after secondary engagement of the T-cell receptor complex with antigen (35,36) or signal-transducing antibodies (7,37), the cells differentiate into immunocompetent T cells. Because only a fraction of the lymphoid cells within TDLNs represent tumor-reactive pre-effector cells, approaches to enhance the capability of stimulating this subgroup of cells would have potential clinical usefulness.

**TABLE 3.** *In vivo efficacy of MCA 205 TDLN standard with TNFα: effect of IL-10 neutralization*

| | Adoptive Immunotherapy* | | |
|---|---|---|---|
| TDLN cells cultured with | No. cells | Antibody | Mean No. Lung Mets (SEM) |
| A. — | None | None | 205 (2) |
| B. — | None | Rat IgG | 186 (40) |
| C. — | None | anti-IL-10 | 170 (27) |
| D. Standard | $2 \times 10^6$ | Rat IgG | 49 (6)† |
| E. Standard | $2 \times 10^6$ | anti-IL-10 | 29 (9)† |
| F. TNFα | $2 \times 10^6$ | Rat IgG | 22 (3)‡ |
| G. TNFα | $2 \times 10^6$ | anti-IL-10 | 5 (2)§ |

TDLN, tumor-draining lymph nodes; TNF, tumor necrosis factor; SEM, standard error of the mean.

* Mice were inoculated with $3 \times 10^5$ tumor cells intravenously. Three days later some groups of mice received effector cells cultured with or without TNFα (25 ng/mL). All groups of mice received IL-2 as described in Table 1. Mice received rat IgG or anti-IL-10 mAb intravenously daily for 4 days after cell transfer.

† $p < 0.01$ compared to A, B, and C.

‡ $p < 0.01$ compared to A, B, C, and D.

§ $p < 0.05$ compared to A–F.

In this report, we tried to modulate the "effector" phase of the immune response by examining the role of TNF-α on the maturation of sensitized pre-effector cells. Tumor-draining lymph node cells activated with anti-CD3 and IL-2 in the presence of TNF-α yielded effector cells with augmented anti-tumor reactivity in three different tumor models. Importantly, after activation with TNF-α, TDLN cells secreted more IFN-γ on secondary stimulation with tumor antigen or anti-CD3 than did control TDLN cells. As we have previously reported, type 1 cytokines (i.e., IFN-γ) have a critical role in mediating tumor regression, whereas type 2 cytokine (i.e., IL-10) has suppressive functions on the anti-tumor reactivity mediated by adoptively transferred cells. Thus, an immunomodulatory effect of TNF-α appears to result in the differentiation of TDLN cells into type 1 secreting cells. Flow cytometric analysis showed that the modification by TNF-α elicited not only enhanced type 1 cytokine secretion per cell but also increased the number of cells expressing this function.

We used human recombinant TNF-α as an adjuvant for T-cell activation. Human recombinant TNF-α is cross-reactive between mice and humans (38). Furthermore, it has been reported that human recombinant TNF-α is fully reactive on murine TNF-1 but not TNF-2 receptors (39). Thus, the effect of human recombinant TNF-α used in this model appears to be mediated through TNF-1 receptors on T cells. Several reports have documented the immunomodulatory role of TNF-α on T-cell anti-tumor function. Scheurich et al. (40) observed that TNF-α exposure to activated human T cells resulted in increased expression of HLA-DR molecules and high-affinity IL-2 receptors that allowed enhanced proliferation to IL-2. Furthermore, in their study, TNF-α was found to be a costimulator of IL-2–dependent IFN-γ production. Lu et al. (41) reported that the in vitro culture of activated human T cells with TNF-α synergized with

IFN-γ to mediate the release of granulocyte–macrophage colony-stimulating factor and granulocyte colony-stimulating factor. In our murine studies, we observed increased granulocyte–macrophage colony-stimulating factor release from TNF-α–cultured TDLN cells (data not shown). These reports clearly document that TNF-α can upregulate the growth and functional activities of activated T cells.

A possible mechanism by which TNF-α upregulates the tumor reactivity of TDLN cells may be its effect on dendritic cells (DC). Freshly harvested TDLN cells include DCs as antigen-presenting cells. Approximately 1% of TDLN cells are DCs, as confirmed by double-positive staining of CD11c and CD80 or MHC class II and CD86 in flow cytometric analysis (data not shown). Dendritic cells are potent immune stimulator cells that can induce strong alloresponses at DC:T cell ratios as low as 1:100. Tumor necrosis factor-α has been shown to enhance the function of DC-presenting soluble antigen to $CD4^+$ T cells in vitro and to increase their yield (42). The concentrations of TNF-α in the latter study (i.e., 10 ng/mL) were within the range used in our studies (i.e., 25 ng/mL). Thus, some of the modulatory effects of TNF-α on TDLN cells may be to enhance the quantity and quality of antigen presentation by DC cells.

Our findings suggest that in vitro TNF-α exposure of TDLN cells promotes a type 1 immune response, and downregulates a type 2 response. We have previously reported that type 2 immune responses in which IL-10 is secreted after antigen stimulation can suppress tumor regression mediated by the adoptive transfer of TDLN cells (11). The downregulation of IL-10 production by TDLN cells resulting from TNF-α culture may be another mechanism by which effector T-cell function is enhanced. Nevertheless, the low levels of IL-10 released by the TNF-α–cultured TDLN cells still had significant suppressive abilities. This was evident in the experiment in which we neutralized the effect of IL-10 by administering anti–IL-10 mAb. In that experiment, the therapeutic efficacy of TNF-α–cultured TDLN cells was further augmented. Our current, ongoing efforts are to determine if we can separate out subpopulations of TDLN cells based on adhesion markers that appear to segregate type 1 from type 2 effector T cells.

In conclusion, TNF-α can modulate the in vitro maturation of pre-effector T cells into immunocompetent T effector cells. Tumor necrosis factor-α–cultured TDLN cells have an enhanced ability to mediate tumor regression on adoptive transfer. Our findings have relevance to clinical efforts to generate tumor-reactive T cells for cancer therapy.

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